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Activin A serum levels and aging of the pituitary-gonadal axis: a cross-sectional study in middle-aged and elderly healthy subjects

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Abstract

Aim of the study was to investigate activin A serum concentration in healthy adult males and postmenopausal females over a wide age-range and its correlation to gonadotropins, inhibin B and testosterone concentrations. The study included 73 males (aged 30–101 years) and 42 postmenopausal females (aged 50–104 years). Blood samples were collected after an overnight fast to measure serum activin A, inhibin B, LH, FSH, and gonadal steroid levels. A significant increase in serum activin A levels over age in both genders, especially in the oldest age-groups, was observed. Serum inhibin B and testosterone concentrations showed a sharp decrease in male subjects, reflecting the age-related decrease of testicular function and by consequence serum FSH and LH significantly increased. In female subjects LH and FSH levels were very high in subjects in their 50s and showed a continuous decline due to pituitary aging. Simple and multivariable regression analyses demonstrated the lack of correlation between activin A and FSH in both males and females. In conclusion, a steep increase in activin A levels is present during aging in both genders, especially in the last decades of life. The physiologic role and site of production of activin A in old subjects remain to be clarified.

Keywords: Activin A; Inhibin B; Aging; Pituitary-gonadal axis; Gonadotropins

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1. Introduction

Pituitary gonadotropins are the major hormones acting on the gonads and their secretion is finely regulated by stimulatory and inhibitory factors, among the latter the feedback mechanisms being predominant. Inhibins and activins are structurally related dimeric proteins with the ability to affect FSH secretion from pituitary gland (Vale et al., 1986, 1988). Inhibins selectively suppress FSH secretion (Scwall et al., 1989), whereas activins are supposed to stimulate FSH secretion (Scwall et al., 1989). Inhibin A is an $\alpha-\beta_A$ dimer and inhibin B is an $\alpha-\beta_B$ dimer, while activins are homodimeric proteins ($\beta_A-\beta_A$ for activin A, $\beta_A-\beta_B$ for activin AB and $\beta_B-\beta_B$ for activin B). The above subunits share structural similarities with the proteins of the transforming-growth-factor β (TGF-β) superfamily, which presently encompasses about 35 different members (Dube et al., 1998).

Although it was suggested that gonadal tissue is the primary site of activin and inhibin production in vivo, several extragonadal sources have been subsequently identified (Meunier et al., 1988). Activin and inhibin bind at different affinity to a common receptor named activin II receptor (ActRII), thus activating or inhibiting its serine/threonine kinase-mediated transduction pathway, respectively (Lebrun and Vale, 1997; Lewis et al., 2000).

It has recently been shown that serum activin A in male healthy subjects increase with age up to 60 years, whereas no variation in activin A levels has been reported in post-menopausal women (Loria et al., 1998). However, whether serum activin A concentrations varies in older ages and, particularly, in the latest decades of life is unknown. Aim of the present study was to evaluate serum activin A levels in healthy adult males and post-menopausal females over a wide age-range and to investigate its correlation to serum FSH, LH, inhibin B and gonadal steroid levels.

2. Subjects and methods

2.1. Subjects

The study included 73 males aged between 30 and 101 years and 42 postmenopausal females aged between 50 and 104 years. Admittance criteria for the study excluded subjects with any medical illness, including obesity and thyroid disease, or serious illness such as cancer, myocardial infarction, pulmonary, renal and hepatic disease within the previous year, as well as those using estrogen, antipsycotropic or other medications known to affect pituitary hormone secretion. Overall the subjects were considered to be in better-than-average health. All women reported the absence of menses for more than one year, with history of previous regular menstrual cycle. All subjects gave their informed consent to the study. The study protocol was approved by local ethic committee. Blood samples were collected after an overnight fast. After collection, samples were centrifuged, aliquoted and stored at $-20°C$ until assay.

2.2. Hormone measurements

Serum activin A and inhibin B were evaluated by solid phase sandwich ELISA kits

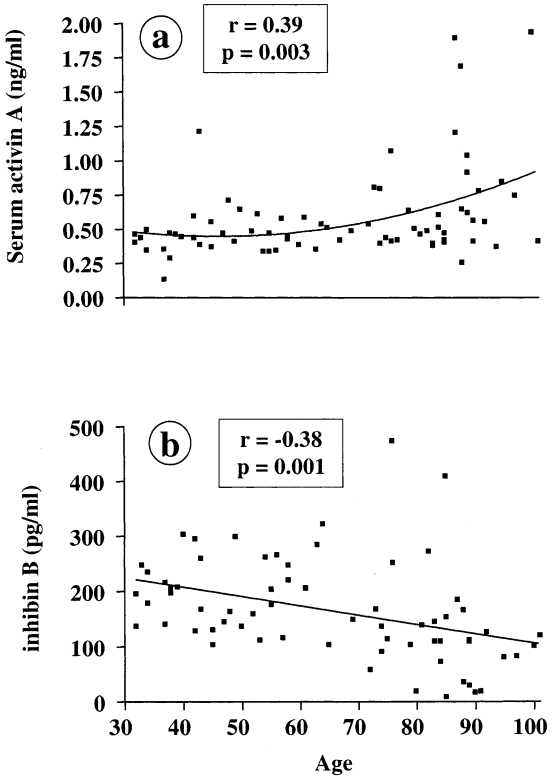

Fig. 1. Serum levels of activin A (a) and inhibin B (b) in 73 healthy males aged between 30 and 101 years.

(Serotec, Oxford, UK) with a sensitivity of 0.1 ng/ml for activin A and 15 pg/ml for inhibin B. The intra- and interassay coefficients of variations were <7 and <10%, respectively. The assay of activin A had no detectable cross-reaction with inhibin A, inhibin B, activin B or follistatin, and a cross-reactivity of <5% with activin AB. The assay of inhibin B had a cross-reactivity of 1% with inhibin A. Serum LH, FSH and testosterone were measured by IFMA commercial Kits (Delfia EG & G, Wallac) with a sensitivity of 0.05 U/L, 0,05 U/L and 0,4 nmol/l, respectively. The intra- and interassay coefficients of variation were 4.5% and 9.5% for LH and 5.2% and 7.9% for FSH and 4.2% and 5.3% for testosterone, respectively. Since serum inhibin B and 17-β-estradiol are known to be very low in post-menopausal women, their concentrations were not measured in female subjects.

2.3. Statistical analysis

Data are expressed as mean ± SD. Non-parametric modified Kruskal–Wallis test for trend was used to assess differences across age groups (Cuzick, 1985). Differences were considered statistically significant if $P < 0.05$. Simple linear or polynomial regression

Table 1
Serum mean (SD) levels of activin A, inhibin B, FSH, LH, and testosterone in healthy male subjects, by age groups

	30–50 years ($n = 20$)	50–65 years ($n = 14$)	65–80 years ($n = 12$)	80–90 years ($n = 18$)	90–101 years ($n = 9$)	Test for trend[a]
Activin A (ng/ml)	0.47 (0.21)	0.47 (0.11)	0.58 (0.21)	0.72 (0.46)	0.74 (0.48)	$P < 0.01$
Inhibin B (pg/ml)	198 (60)	209 (66)	165 (121)	129 (101)	78 (45)	$P < 0.0005$
FSH (U/l)	4.8 (3.5)	6.5 (4.5)	12.0 (9.5)	19.9 (14.4)	22.2 (16.4)	$P < 0.0001$
LH (U/l)	2.9 (1.0)	4.0 (1.6)	8.2 (6.2)	10.2 (9.2)	16.1 (13.3)	$P < 0.0001$
Testosterone (nmol/l)	20.7 (6.7)	19.6 (5.8)	15.0 (7.8)	9.4 (5.8)	9.2 (6.5)	$P < 0.0001$

[a] Non-parametric test for trend across age-groups.

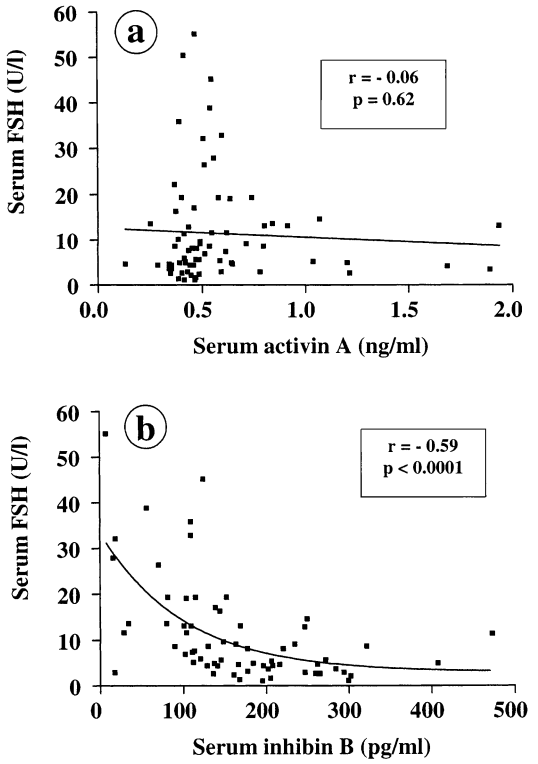

Fig. 2. Correlation of activin A (a) and inhibin B (b) serum levels to serum FSH levels in male subjects.

analysis was also used to test for correlation between hormonal parameters, as appropriate. Pearson's product-moment correlation coefficients (r) are reported. Multivariable regression models were used to simultaneously evaluate the association of activin A, inhibin B, testosterone, LH, and age to serum FSH levels. FSH was inserted in the model as dependent variable after logarithmic transformation to accomplish normal distribution requirement. Model validity was evaluated by graphical inspection of standardized and jackknifed residuals.

3. Results

3.1. Males

As shown in Fig. 1a, male subjects exhibited a continuous increase of activin A serum levels over age ($r = 0.39$, $P = 0.003$). Activin A rose from 0.47 ± 0.21 ng/ml in subjects aged 30–50 years to 0.74 ± 0.48 ng/ml in those aged 90–101 years, paralleling FSH increase across age-groups (Table 1). However, in linear regression analysis activin A and FSH serum levels did not show any correlation ($r = -0.06$, $P = 0.62$; Fig. 2a). Serum

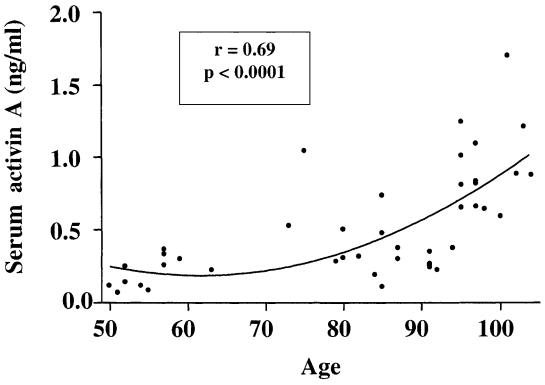

Fig. 3. Serum levels of activin A in 42 postmenopausal healthy females aged between 50 and 104 years.

inhibin B fell from 198 ± 60 pg/ml in the 30–50 years group to 78 ± 45 pg/ml in the 90–101 years group (test for trend across age-groups, $P < 0.001$, Table 1). As expected, inhibin B appeared to be inversely associated to serum FSH ($r = -0.59$; $P < 0.0001$), as shown in Fig. 2b. Serum LH levels showed increased over age from 2.9 ± 1.0 U/L in subjects aged 30–50 years to 16.1 ± 13.3 U/L in subjects aged 90–101 years ($P < 0.0001$, test for trend across age groups). This increase has to be ascribed to the concurrent decrease of testosterone secretion (Table 1), which is known to exert a negative feedback on pituitary secretion of LH.

In order to further investigate the hormonal interrelations involved in FSH secretion control, we performed a multi-variable regression analysis that confirmed inhibin B to be an independent determinant of FSH levels ($P < 0.001$) after adjustment for age, activin A, LH, and testosterone. Even in this analysis serum FSH was not related to activin A levels ($P = 0.72$). In the model, the statistical interaction between serum activin A and inhibin B levels was not significant ($P = 0.31$), suggesting that the relative concentrations of activin A and inhibin B (activin A/inhibin B ratio) may not play any role in FSH serum-level control. Finally, the model demonstrated that the age-related increase of FSH was significant ($P < 0.0001$) also after adjusting for activin A, inhibin B, LH, and testosterone.

3.2. Females

Serum activin A exhibited in post-menopausal healthy females a significant age-related increase ($r = 0.69$, $P < 0.0001$; Fig. 3). The highest increase of activin A was found in the oldest decades of life, when activin A heightened from 0.55 ± 0.37 ng/ml in the 90–95 years group to 0.94 ± 0.33 ng/ml in the subjects between age 95 and 104 (Table 2). This rise was accompanied by a sharp decline in both FSH and LH levels (Table 2). Although activin A was negatively associated with FSH in linear regression analysis ($r = 0.41$, $P < 0.01$, Table 2), no association was found between FSH and activin A serum levels after adjustment in multi-variable regression analysis for age and LH serum levels ($P = 0.96$). The regression model used also confirmed the independent effect of age on serum FSH levels ($P < 0.01$).

Table 2
Serum mean (SD) levels of activin A, FSH, and LH in healthy female subjects, by age groups

	50–65 years ($n = 11$)	65–90 years ($n = 12$)	90–95 years ($n = 10$)	95—104 years ($n = 10$)	Test for trend[a]
Activin A (ng/ml)	0.21 (0.10)	0.43 (0.25)	0.55 (0.37)	0.93 (0.33)	$P < 0.0001$
FSH (U/l)	71.0 (24.8)	55.9 (30.2)	45.2 (20.2)	25.9 (23.5)	$P = 0.0001$
LH (U/l)	29.1 (8.4)	25.1 (13.9)	19.6 (12.7)	9.9 (9.9)	$P < 0.0005$

[a] Non-parametric test for trend across age-groups.

4. Discussion

Although previous studies showed age-related differences in activin A levels (Loria et al., 1998; Harada et al., 1996), little information are available on the oldest decades of life. Our study investigated for the first time healthy male and female subjects over a wide range of age, including also a sizeable number of centenarians. By enrolling healthy oldest subjects, the study may have been hampered by selection bias, in particular age-related self-selection as the older subjects are likely to be different to many respects from the subjects who do not survive until such an advanced age. However, the cohort-study design may not practically be proposed to investigate such a large age-range, which included also centenarians. In addition, Loria et al. did not found any substantial discrepancy between cross-sectional and cohort design as they longitudinally evaluated activin A in a small group of subjects (Loria et al., 1998). Moreover, for a bias to result from age-related selection we should suppose that the levels of activin A are significantly related to subject survival. In the present study we demonstrated a significant increase in serum activin A levels over age in both genders, which was particularly apparent in the oldest age-groups. Our results confirm the report of Loria et al. of an activin A positive trend in male subjects aged between 20 and 60 years (Loria et al., 1998). However, the extension of the age-range provided by the present study allowed to demonstrate a sharp rise of serum activin A concentrations in the last years of life. Moreover, by including a group of oldest women in the study, it was possible to demonstrate an age-related increase of activin A also in female sex which was not found in a previous study considering a narrower age-span (Loria et al., 1998).

Although in vitro and animal studies showed the stimulatory effect of activin A on FSH secretion (Scwall et al., 1989), we were unable to find any positive correlation between serum activin A and FSH levels both in male and female subjects. In particular, although in males the increase of activin A over age paralleled FSH rise, in linear regression analysis these two parameters did not show any correlation. By contrast, inhibin B, whose serum levels decreased with age probably reflecting the steady decline in testicular function occurring during aging (Mahmoud et al., 2000; Byrd et al., 1998; MacNaughton et al., 1991; Tenover et al., 1988), appeared to be inversely associated to serum FSH both in simple than in multi-variable regression analyses. In the present study inhibin B resulted to be an independent determinant of FSH after adjustment for age, activin A, LH and testosterone. Moreover, although it has been suggested that FSH increases during aging may result from a higher activin A/inhibin B ratio (Veldhuis et al., 1999), this hypothesis was not supported by our data showing a lack of significant interaction between the two subunits.

In the oldest decades of life healthy females showed a sharp decline in serum gonadotropins probably related to the aging of the pituitary gland (Lamberts et al., 1997; Vaninetti et al., 2000). In this study a rather surprising negative correlation between activin A increase and FSH decrease was observed in simple regression analyses. However, as likely in a cross-sectional study with large differences in subjects' age, those results were heavily confounded by the association of both activin A and FSH serum levels with age. For those reasons we carried out multi-variable regression analyses on our data that, after adjustment for age, as well as for the other hormones studied, did not show any effect of activin A on FSH serum concentrations also in females.

The steep increase of activin A observed in the oldest subjects of both sexes lead to convincingly exclude gonads as a valuable source of its secretion in the old subject. Moreover, the absence of correlation of serum activin A to FSH induces to argue that the function of activin A in the old subject may outrun the traditional endocrine role in FSH control (Welt and Crowley, 1998). Indeed, activin A shows a diffuse expression, having been identified in tissues as diverse as placenta, bone marrow, brain, and endothelium (Meunier et al., 1988). Recently, activin A expression in atherosclerotic lesions in humans has been reported, and its role in plaque stabilization has been proposed (Engelse et al., 1999), being activin A a potent mitogenic co-stimulator of vascular smooth muscle cells (Molloy et al., 1999). This observation may explain the steady increase of activin A we observed during aging. Furthermore, activins could play a role in tissue repair and it may exert neuroprotective and anti-inflammatory activities (Munz et al., 1999). The demonstration of high levels of circulating activin A in oldest subjects of both sexes may foster the development of research on the role of this multifunctional protein in the aging processes.

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